Differences of secondary metabolites content in Kasturi Mango (*Mangifera casturi* Kosterm.) from South Kalimantan by the application of artificial light using light emitting diode

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Abstract. Mango (*Mangifera casturi* Kosterm.) is one of the endemic plants of South Kalimantan, which is known to have a long juvenile phase. Applying light to plants will affect plant morphology and physiology changes. The provision of LED light as artificial light is known to modify plant metabolites. This research aims to determine changes in the response effects of the addition of artificial light using red, blue, and white monochromatic LEDs on secondary metabolite profiles in Kasturi mango seedlings from three Kasturi accessions: Mawar, Pelipisan, and Kasturi. Secondary metabolites were measured using the GC/MS and LC/MS methods. Profiling of metabolites using the GC/MS method resulted in 18 secondary metabolites, with the common compounds belonging to the sesquiterpenoid group. The Pelipisan accession contains unique compounds from the steroid group with different abundances for each type of LED lamp used. Estimating secondary metabolites using the LC/MS method obtained results of 10 compounds consisting of alkaloids, phenolics, antioxidants, and flavonoids.

1 Introduction

Light have been plays as an essential role in the ecology and physiology of plants. Plants use the light as a source of energy for growth and their use for photosynthesis and also source of information to external and internal protect from unfavorable environment to support their growth and development. The frequency of lighting from the sun in the form of time, intensity, and irradiation spectrum will signal plants to regulate seasonal phenology and variations in phenotypic expressions, including forms of growth and allocation of sources [1]. The administration of artificial light in changes in several wavelengths and a combination of the light spectrum will affect the changes in plants morphological and physiological development. The artificial light in plant cultivation sources that is often used in research is Light Emitting Diode (LED) because LED lights provide a maximum value of Photosynthetic

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Active Radiation (PAR) efficiency ranging from 80-100% [2]. LEDs light facilitates the intensity, spectrum, and energy arrangements and can also be used for secondary metabolite manipulation in target plants to increase plant productivity and quality. LEDs often influence the response of light on plants, which are red, blue, and white. This light generally induces hormones and compounds such as auxin, governor biosynthesis, flavonoids, ascorbic acid compounds, and defense genes [3]. Giving blue or red LED lights can increase antioxidant and phenolic content in leaf vegetables and increase the synthesis of volatile compounds in tomatoes using red/blue LEDs, affecting tomatoes' sweet taste [4].

Mangifera casturi Kosterm. or Kasturi mango is fruit trees and one of the mango from South Kalimantan originating from the crossing of *Mangifera indica* x *Mangifera quadrifida*. This mango has several commonly found types: Kasturi, Pelipisan, Cuban, and Pinari [5]. Kasturi mango is a fruit with a small fruit size, with the color of a skin of the fruit from orange-yellow to blackish purple, the taste of sweet fruit with a strong fragrance and fibrous flesh [6]. Kasturi mango has other economic value that can be utilized, namely some metabolite compounds that can be extracted from fruit, leaves, and bark, which can benefit humans. The mango leaves contain tannin compounds, flavonoids, and triterpenoids [7]. The bark contains terpenoids, steroids, and saponins, and the content of methanol extracts from kasturi contain has been metabolite compounds which have bioactive compounds such as terpenoids, steroids, phenolics, and flavonoids [8], and its factions contain anti-bacterial compounds [9]. Metabolite compounds in plants can also indicate plant phase changes based on the type and content [10].

Kasturi mango is known to have a long enough juvenile phase to arrive at the generative phase, which takes up to 25 years [11]. This has become one of the problems faced in cultivating Kasturi manga. The treatment of the use of the LED spectrum is known to affect morphogenesis and plant physiology and can increase metabolism and stimulate flowering [12]. Mango Kasturi has the potential to be developed apart from using the fruit. Therefore, it is necessary to have the right cultivation activities to optimize the results. One of the beginnings of this activity was to use good-quality seeds. In the process of adding artificial light to Kasturi mango seedlings, it is expected that compounds are related to changes in plants responses during the addition of LEDs [13]. This research is expected to be able to find out the changes in the morphophysiological response with the addition of artificial light from LEDs on the content of secondary metabolites in Kasturi mango seedlings and their relationship to the growth and development of Kasturi mango seedlings with a shorter plant juvenile period.

2 Material and methods

The research was conducted from January to June 2021 at Screenhouse (shading net 90%), Leuwikopo Experimental Garden, Department of Agronomy and Horticulture, IPB University. The research was designed using the design of a tangled plot with two factors, namely LED lights as the main plot (red LED, white LEDs, and blue LEDs) and Kasturi mango accessions as the nested factors (Cuban accession, Pelipisan accession, and Kasturi accessions). Research materials use Kasturi mango seeds from 3 accessions, namely Cuban, Pelipisan, and Kasturi, which originated in South Kalimantan. The nursery was held in January 2020. The seeds were one year old and stored in nurseries using 90% paranet. The installation of the lamp is carried out, the distance between the canopy and the lamps of 45 cm. LED lights used are the 60-eye Module SMD 5050, with red, white, and blue colors. Treatment of plant lighting conditions arranged for 20 hours (04.30-00.30) and the dark conditions for plant is 4 hours (00.30-04.30).

2.1 Secondary metabolites preparation using GC/MS method

The measurement of secondary metabolites using GC/MS method is carried out two months after the LED treatment. The number of samples measured is taken by composite on each seed and each treatment. The leaves are taken under the top leaves but still receive light directly. The leaves are separated from the leaf bone and cut into small pieces. They are stored in the conical tube and then frozen to maintain the freshness of sample were used. Sample preparation was carried out before the substance injection process to each tool. The sample is then dried in the oven for approximately 3 hours until completely dry. Dry samples were crushed with a blender until smooth, and then maceration was carried out with ethanol for 3-5 hours. The sample that has been macerated is then put into a conical tube and dried at 60 °C for 1 hour. After drying, dissolve again with the remaining 200 µl maceration extract, and the results are injected into GC-MS machine [14].

2.2 Secondary metabolites preparation using LC/MS method

The measurement of secondary metabolites using the LC/MS method is carried out two months after the LED treatment. The number of samples measured is taken by composite on each seed and each treatment. The leaves used are separated from the leaf bones, cut into small pieces, stored in the conical tube, and frozen to maintain the freshness of the sample. The sample preparation is carried out before the substance injection process to each tool. The sample is then dried in the oven for approximately 3 hours until completely dry. LC/MS analysis uses the Shimadzu LC-IT-TOF QTOF system with positive ionization mode. The ESI parameter includes a capillary temperature of 120°C and 500 l $hour^{-1}$ powder gas, 4.5 kV voltage source. Ion scanning mode from M/Z 100-2000 was carried out with a 200°C source, and also the column of temperature must be maintained at 40°C. The motion phases in elusion binary (1) 5 mm (NH_4) OA_C solids and (2) the condition of the gradient 0-30 minutes and followed by CH_3CN, a linear gradient of 10%-100% B, 30-40 minutes isocratic at 100% B. Flow rate 0.2 ml $minutes^{-1}$. In MS/MS step, the Spectrophotometer analysis was setting to survey ions in the M/Z 100-1000 range [15]. Chromatogram data processing based on the estimation results uses the M/Z value or an accurate mass at the peak of the chromatogram, which is then validated with an online database using data from Massbank (https://masbank.eu/massbank/).

3 Results

Profiling of secondary metabolites using the GC/MS method on the leaves of the Kasturi mango seedlings to find 18 compounds. This metabolitic compound is classified into several compounds, most of the sesquiterpenoid groups, followed by steroids, antioxidants, and alkaloids. Characteristics and abundance of secondary metabolitic compounds in kasturi mango leaves with GCMS (Table 1).

Table 1. Content of secondary metabolite compound from Kasturi mango with the GC/MS method

RT	Compounds	Abundance (%)						Grups compunds
		PR	KR	PW	KW	PB	KB	
10,72	Tricyclo[4,4,0,0(2,7)]dec-3-ene, 1,3-dimethyl-8-(1-methyl ethyl)-, st	1,32	0,39	,	,	,	,	Terpenoids

Table 1. Content of secondary metabolite compound from Kasturi mango with the GC/MS method (continued)

RT	Compounds	Abundance (%)						Grups compunds
		PR	KR	PW	KW	PB	KB	
11,65	1,1,4,7-tetramethyl-1a,2,3,4,4a,5,6,7b-octahydro-1h-cyclopropa[e]azulene	0,36	,	,	,	,	,	Sesquiterpenoids
12,14	4,11,11-Trimethyl-8-methylenebicyclo[7,2,0]undec-4-ene	2,67	1,13	,	,	,	,	Sesquiterpenoids
13,52	1,1,7-Trimethyl-4-methylenedecahydro-1h-cyclopropa[e]azulene	3,27	1,29	,	,	,	,	Sesquiterpenoids
13,54	1H-Cycloprop[e]azulene, decahydro-1,1,7-trimethyl-4-methylene-, [1aR-(1a,alpha,4a,beta,7,alpha,7a,beta,7b,alpha,)]-	,	0,77	,	,	,	,	Sesquiterpenoids
14,34	8-Isopropyl-1-methyl-5-methylene-1,6-cyclodecadiene	0,64	,	,	,	,	,	Sesquiterpenoids
14,80	Naphthalene, 1,2,3,4,4a,5,6,8a-octahydro-4a,8-dimethyl-2-(1-methylethenyl)-, [2R-(2,alpha,4a,alpha,8a,beta,)]-	,	,	,	3,59	,	,	Sesquiterpenoids
26,49	11-Tetradecyn-1-ol acetate	,	,	,	,	,	0,68	Antioxidant
26,51	Bicyclo[3,1,1]heptane, 2,6,6-trimethyl-	0,62	,	,	,	,	,	Monoterpenoids

Table 1. Content of secondary metabolite compound from Kasturi mango with the GC/MS method (continued)

RT	Compounds	Abundance (%)						Grups compunds
		PR	KR	PW	KW	PB	KB	
26,72	1,2,3-Benzenetriol-	,	,	,	2,34	,	,	Antioxidant
27,14	1,1'-Biphenyl, 2,2'-dimethyl-	0,57	,	,	,	,	,	Benzene
27,71	benzocyclooctene, 6,8-dimethyl-f	,	5,72	,	,	,	,	Alkaloid
33,12	(2r,3s)-3-bromo-2-ethyl-8-(1'-bromohexyl)oxocane	,	,	,	,	,	62,32	Pheromone
34,35	C(14a)-homo-27-nor-14,beta,-gammaceran-3,alpha,-ol	,	,	,	94	,	,	Antioxidant
40,91	Isoaromadendrene epoxide-	,	90,7	,	,	,	-	Sesquiterpenoids
45,97	Pregn-5-en-3-ol, 21-bromo-20-methyl-, (3, beta,)-	47,9	,	100	,	100		Steroids
48,47	Ergost-25-ene-3,5,6-triol, (3,beta,5,alpha,6,beta,)	,	,	,	,	,	37	Steroids
49,02	9,19-Cyclolanostan-3-ol, 24-methylene-, (3, beta,)	42,7	,	,	,	,	,	Steroids

RT: Retention Time, PR: Pelipisan Red LED, KR: Kasturi Red LED, PW: Pelipisan White LED, KW: Kasturi White LED, PB: Pelipisan Blue LED, KB: Kasturi Blue LED.

Secondary metabolitic profile analysis on Kasturi mango leaves is also carried out using the LC/MS analysis method. The number of compounds identified with LCMS is 13 compounds, relatively less than GC/MS. Characteristics of secondary metabolitic compounds and compounds in Kasturi mango leaves identified using LCMS (Table 2).

Table 2. Prediction of secondary metabolites on Kasturi mango leaves under LED treatment using the LC/MS method

LED treatment	Accession	RT	Compounds	m/z	Groups compounds
Red LED	Kasturi	1,53	Pseudojervine	382,33	Glikosida

Table 2. Prediction of secondary metabolites on Kasturi mango leaves under LED treatment using the LC/MS method (continued)

LED treatment	Accession	RT	Compounds	m/z	Groups compounds
Red LED	Kasturi	1,53	Pseudojervine	382,33	Glikosida
		16,44	Phosphatidylcholine 15:0-16:1	702,43	Phospolipid
		20,18	Isoplumericin	259,63	glukosida
	Pelipisan	1,53	Pseudojervine	382,36	Glikosida
		13,29	Vitamin P, bioflavonoid	546,43	Flavonoid
		16,44	Voacamine	702,43	Alkaloid
		20,18	Salicylic acid	259,25	Fitohormon
White LED	Pelipisan	1,53	Pseudojervine	105,33	Glikosida
		4,17	Luteolin-6-C-glucoside	528,55	Flavonoid
		10,99	Mangiferin	702,80	Flavonoid
		12,35	Isoharmnetin-3-glucoside-4'-glucoside	538,46	Glikosida
		14,31	Voacamine	702,36	Alkaloid
	Kasturi	1,53	gALnAFUgICnACtHRnAC ii	382,29	Antioksidan
		4,17	Isohemandezine	374,33	Alkaloid
		15,16	Phosphatidylcholine 15:0-16:1	460,12	Phospholipid
		16,44	Voacamine	476,30	Alkaloid
		20,18	Bioflavonoid-flavone base +3MeO and flavone base + 3MeO	702,36	Flavonoid
Blue LED	Kasturi	1,53	Pseudojervine	382,23	Glikosida
		4,34	Vocamine	105,33	Alkaloid
		16,44	Subsessiline	352,32	Fenol
	Pelipisan	20,18	L-Glutathione (*oxidized form*)	328,23	Antioksidan
		1,45	Pseudojervine	382,42	Glikosida
		15,16	Isohemandezine	528,18	Alkaloid
		16,35	Vocamine	702,49	Alkaloid
		20,35	N-Fructosyl allanin	322,47	Fenolik

4 Discussion

The group of sesquiterpenoid compounds (Table 1) found is generally volatile, while in white castles, antioxidant compounds that act as scavenging when ROS occurs are found,

such as the pyrogallol group. In Pelipisan, there are specific compounds in the steroid group where the administration of white and blue light produces 100% abundance, and red is only 42.56%. Sesquiterpenoid compounds were found in the red LED treatment in this study. Sesquiterpenoid compounds are common compounds that appear in the treatment of red light found in chloroplasts and play a role in the prevention of ROS [2]. Red LEDs are also known to affect volatile compounds and antioxidant compounds in basil leaves, where light increases antioxidant capacity in plants in conditions of differences in light intensity [16]. The highest group of compounds is from the Sesquiterpenoid group, a compound with C15 atoms as one component of essential oils that produces aroma in plants such as fruits and leaves.

In addition, this group of compounds can act as a compound to prevent damage caused by ROS in the *Atractylodes lancea* plant [17]. Other groups of most steroids, such as *ergosterol, cytosterol,* and *progesterone,* are known as secondary metabolites, which are amphiphile fat compounds that have a role in regulating cell membrane permeability and absorption in fat tissue [18] on plant leaves and also play a role in maintaining leaves to inhibit leaf aging and prevent abscission in leaves [19]. In Kasturi, with the administration of blue light, there are specific compounds of ergosterol steroids that are also found in *Dysoxyllum malabricum*, *Dysoxyllum mollissimum*, and mahogany which are extracted in the form of ethanol and methanol, which have roles as anti-bacterial and anti-microbial [20]. Secondary metabolites also changes as the plant grows as form the transition from vegetative to generative phases or juvenile period. The effect after LED treatment basically giving the responses to plant. In experiment it is known that the red LED treatment shows a high wavelength and has a higher brightness scale than the treatment of blue LED light [21]. The intensity of lighting also given an affects the plants growth and development of vegetative phases. The higher the brightness scale, the higher the wavelength produced and will affect the secondary metabolites produced by plants and respond to existing environment [22].

Variations of LC/MS data (Table 2) can be caused by biological variations, which can also be caused by analytic reasons such as sample preparation. The condition of the instrument or environmental operation in data accuracy, variations are seen from the time of retention, an increase in the intensity of the value and value of M/Z [23]. The obtained metabolite compounds are included in several groups, including alkaloid compounds, phospholipids, flavonoids, antioxidants, and phytohormones. In this study, the general compound found was from the type of pseudojervine from the glycoside group. This pseudojervine compound is commonly found in several types of plants, such as *Veratrum californicum*. Alkaloids are the most commonly found compounds in every LED and accession treatment.

One of the group of secondary metabolite compounds commonly extracted and found in plants other than terpenoids and phenol compounds are alkaloids [24]. Alkaloids are secondary metabolite compounds that also have an N atom. Some types of alkaloids have additional oxygen, sulfur, chlorine, bromine, and phosphorus. Alkaloid biosynthesis in plants can be influenced by plant stress triggered by the environment, such as pathogens, fungi, heavy metal content, light, and osmotic stress [25]. Light induces the enzyme and its activation, as in the *Catharanthus roseus* plant. Light induces the biosynthesis of vindolin and acid compounds, such as physical or salicylic acid, due to the peroxidase activity in plants. Plants illuminated by ultraviolet light B continuously increase the content of vindolin in *Catharanthus roseus* [26]. In some studies, it is known that alkaloid compounds are toxic compounds for pathogens that attack and are defense compounds that are driven by changes in physical acid content in plants [27]. Alkaloids generally accumulate in cell specifics with plants cytotoxicity and defense response capabilities. Compartments of alkaloid compounds and types of alkaloids produced by plants cannot be known with certainty because they will differ in each type of plant and type of stress that occurs [28]. In this study, the possibility of

a common compound from the alkaloid group is the effect of environmental stress that causes plants to produce alkaloid compounds.

Salicylic acid or salicic acid is a phytohormone that plants can produce in response to environmental changes. Salicylic acid as a secondary metabolite in plants indicates plant stress that can be caused by biotic and abiotic stress [29]. Giving continuous light can also cause plants to experience stress. The salicylic acid content in plant tissue is essential because salicylic acid plays a role in optimizing photosynthesis in conditions of plant growth under different light [30]. Salicylic acid plays a role in preventing photodamage in mandarin satsuma leaves [31]. Salicylic acid is generally produced in chloroplasts to protect leaf pigment and induce enzymes and enzymatic compounds that act as plant antioxidants [32].

Secondary metabolites also changes as the plant grows as form the transition of juvenile period into generative phases. The effect after LED treatment its giving the responses to plant. In some experiment, it is known that the phenylpropanoid compound will change its abundance during the organogenesis of plants and changes in plant age, as in sandalwood trees, these compounds are stored as glycosides in vacuoles [33]. Phenylpropanoid compounds require carbon compounds to be synthesized into aromatic amino acid compounds in plants, such as phenylanine, tyrosine, and tryptophan. LED irradiation affects the content of simple sugars such as glucose and fructose and the phenylpropanoid group's compound content in buckwheat plants [34]. Glucosinolate content is another compound that can be a marker that the plant has entered the adult phase. This glucosinolate has a pattern of different compounds during plants ontogenesis in plant tissues and organs. The content of glucosinolate can also be influenced by environmental factors, one of which is the length of the day and the quality of the light used in *Nasturtium officinale,* where the glucosinolate content will increase in the length of treatment of the length [35]. In this study, the content of phenylpropanoid or glucosinolate compounds was not found at the time of the metabolite profiling was possible because the use of Photosynthesis Flux Density (PPFD) Photon values that could not stimulate the changes in the metabolite or these compounds were still in low abundance conditions that caused not to be detected by the device. Phenylpropanoid, which is stored in the form of glycosides in plants, also allows several compounds resulting from profiling using the LC/MS method as a marker of changes in the plant phase in the Kasturi mango seedlings with the calculating abundance of compounds.

5 Conclusion

Profiling metabolite compounds given the respond to metabolite compounds and produce 18 secondary metabolite compounds, with general compounds comprising sesquiterpenoid groups. Pelipisan accession contains special metabolite compounds from the steroid group with different abundances in LED lights applied to plants. Estimation of secondary metabolite compounds using the LC/MS method resulted in 10 suspected compounds consisting of phenolics, flavonoids, antioxidants and alkaloids. The compound estimation in this research has not been able to find specific compounds that are markers of the transition cycle from the juvenile phase to the generative phase in Kasturi mango seedlings based on the response given by the plant.

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